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Spontaneous preterm birth in African Americans is associated with infection and inflammatory response gene variants

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Abstract

Objective—To study the genetic risk factors of spontaneous preterm birth (PTB) in African Americans.

Study Design—Case-control analyses were performed using maternal and fetal DNA from 279 African American birth-events (82 PTB and 197 term) and 1432 single nucleotide polymorphisms (SNPs) from 130 candidate genes. Single locus association and haplotype analyses were performed.

Results—The most significant associations were in the maternal Interleukin-15 (IL-15) (rs10833, allele $p = 2.91\times10^{-4}$, genotype $p = 2.00\times10^{-3}$) gene and the fetal Interleukin-2 Receptor B (IL-2RB) (rs84460, allele $p = 1.37\times10^{-4}$, genotype $p = 6.29\times10^{-4}$) gene. The best models for these markers were additive (rs10833, OR = 0.30, 95% confidence interval (CI) 0.14–0.62, $p = 1.0\times10^{-3}$; rs84460, OR = 2.32, 95% CI 1.47–3.67, $p < 1.0\times10^{-3}$). The largest number of significant associations was found in genes related to infection and inflammation. There were overall a larger number of significant associations in infants than in mothers.

Conclusions—These results support a strong role for genes involved in infection and inflammation in the pathogenesis of PTB, particularly IL-12 and IL-12RB, and indicate that in African Americans there may be complementarity of maternal and fetal genetic risks for PTB.

Keywords

Complex disease; genetic epidemiology; reproductive genetics; infection/inflammation

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Introduction

Preterm birth (<37 weeks gestation) accounts for 12.0–13.0% of pregnancies in the United States, and ~17–18% in African Americans [1], and it is a leading cause of neonatal morbidity and mortality [2]. The majority of preterm births result from spontaneous contractions and idiopathic causes (PTB) [3].

Among the known risk factors of PTB is family history, suggesting that there may be a genetic predisposition to PTB [4–7][8]. Twin studies estimate ranges for the heritability of PTB between 20–40% [9;10]. Racial disparity in PTB rate among Caucasians and African Americans also supports a genetic predisposition to PTB. These data indicate that genetic variation may affect PTB susceptibility. In addition, there have been a growing number of reports of association of genetic variants with PTB, but the results have often failed to replicate in subsequent studies[11–16]. This may be due to variation in phenotypic definitions and/or genetic heterogeneity (the existence of different genetic etiologies in different individuals for the same trait), and different study populations[17]. In addition, it is highly unlikely that a single gene mutation can be a causal factor for such a complex phenotype[18–20].

To understand the complex genetic predispostion of PTB and to elucidate the high rate in African Americans, we performed a large-scale PTB candidate gene association study focusing on an African American population. Data from previously published studies in both *in vivo* and *in vitro* human and animal models suggests that four primary pathogenic pathways either independently or through interactions lead to PTB [21]. The four proposed pathways are: 1) activation of maternal or fetal hypothalamic-pituitary-adrenal (HPA) axis; 2) decidual-chorioamniotic infection/inflammation; 3) decidual hemorrhage (abruption) and 4) pathological distention of the uterus [21]. All pathways culminate in a common terminal pathway that causes the release of uterotonins, such as prostaglandins leading to preterm labor and delivery [21]. We examined 1536 single nucleotide polymorphisms (SNPs) in 130 candidate genes from these hypothesized pathways for association with PTB. Given the potential for both maternal and fetal contribution to PTB, both maternal and fetal DNA were analyzed in this case (spontaneous preterm birth) control (term delivery after a normal pregnancy) study. Single locus and haplotype tests of association were performed and genes were grouped according to known biological/biochemical pathways of PTB.

Materials and Methods

Study population

Subjects were recruited at the Centennial Medical Center, Nashville, TN. Institutional Review Boards at TriStar Nashville, TN and Vanderbilt University, Nashville, TN approved this study. All included pregnancies were singleton live births. Subjects were recruited between September 2003 – December 2006 from a total of ~ 11,250 deliveries of which 1550 were preterm births (<37 weeks). Our cohort (spontaneous PTB with no rupture of membranes) consisted of 513 subjects (of all ethnic groups). Seventy six African American subjects (from a total of 134) were included based on our inclusion/exclusion criteria. Race was identified by self-report and a questionnaire that traces ethnicity back two generations

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from the parents. Individuals who had more than one racial group in their ancestry were excluded from the study. We recruited mothers between the ages of 18 and 40 upon admission in hospital and blood was collected at labor. Gestational age was determined by last menstrual period and corroborated by ultrasound dating. In our study, cases (PTB) were defined as having 2 contractions/10 minutes followed by delivery at $<36^{0/7}$ weeks gestation and controls by normal labor and delivery at term $\geq 37^{0/7}$ weeks with no medical or obstetrical complications during pregnancy. A cut-off of $<36^{0/7}$ weeks gestation was used to correct for the lack of precision of measurements of last menstrual dating and ultrasound dating. Subjects with multiple gestations, preeclampsia, preterm premature rupture of the membranes, placental previa, fetal anomalies, gestational diabetes, poly- and oligohydramnios, and other complications such as surgeries during pregnancies were excluded. Small for gestational age (SGA) was not specifically excluded in the ascertainment. DNA was collected from maternal blood and fetal cord blood.

Demographic and clinical characteristics

African American maternal and fetal samples (maternal-76 case and 191 control; fetal - 65 case and 183 control) were included. Demographic and clinical data were obtained from questionnaires and medical records. Demographic and clinical data included gravidity, gestation age (days), gestational weight (grams (g)), APGAR 1, APGAR 5, maternal age (yrs), smoking (%) and measures of socioeconomic status.

DNA sampling and genotyping

DNA was isolated from maternal and fetal blood samples using the Autopure automated system (Gentra Systems (Minneapolis, MN)). A total of 1536 tag single nucleotide polymorphisms (SNPs) were screened in 130 PTB candidate genes (Supplemental Table 1) (4 genes were within 5000 kb of another gene and were analyzed as members of the established candidate). We chose SNPs based on their ability to tag surrounding variants in the CEPH and Yoruba population of the HapMap database (http://www.hapmap.org) using a minor allele frequency (MAF) of 0.07 in CEPH and 0.20 in Yoruba and r^2 >0.80, as our original study consisted of African Americans and Caucasians. Final analyses included 1432 markers after removing monomorphic markers and those that were not successfully genotyped. 94 markers were not successfully genotyped and 10 were monomorhpic. Genotyping was performed by Illumina's GoldenGate genotyping system (http://www.illumina.com/General/pdf/LinkageIV/GOLDENGATE_ASSAY_FINAL.pdf). Both positive and negative controls were included according to Illumina's standard protocol and both maternal and fetal replicates were selected for inclusion on plates. Samples that did not replicate across plates were removed from the analysis.

Bioinformatics Tools

SNPper (http://snpper.chip.org) using NCBI Build 35.1 was used to determine marker positions (bp), marker function, and identify amino acid changes. Kyoto encyclopedia of genes and genomes (KEGG) (http://www.genome.ad.jp/kegg/pathway.html) was used to examine gene ontology and to group genes into biological process pathways.

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Statistical analysis

Clinical and demographic characteristics between cases and controls were compared using Shapiro-Wilks tests of normality on gravidity, gestational age, gestational weight, APGAR 1, and APGAR 5. All measurements deviated significantly from normality; as a result Mann-Whitney two-sample ranksum tests were used to compare case and control groups [22]. Standard t-tests were used to test whether maternal age differed between cases and controls. χ^2 tests were used to test for differences in the counts of smokers and non-smokers between cases and controls. STATA 9.0 statistical software [23] was used for all analyses.

Samples analyzed represent those without Mendelian inconsistencies. Statistical tests for differences in single locus allele and genotype frequencies, for deviations from Hardy Weinberg Equilibrium (HWE) and for measurements of inbreeding coefficients (F) were calculated using Powermarker statistical software [24]. Statistical significance for these analyses was determined using Fishers Exact tests.

Pairwise linkage disequilibrium (LD) was characterized and haplotype frequencies were calculated using Powermarker [24;25] and HaploView [26] statistical software. Standard summary statistics D′ and r^2 were calculated using HaploView [27]. Haplotype blocks were assigned using the D′ confidence interval algorithm created by Gabriel *et al* (2002) [28]. Both Powermarker and HaploView use an EM algorithm to determine haplotype frequency distributions when phase is unknown. The Powermarker haplotype trend analysis was performed for dichotomous outcome with 2, 3 and 4 marker sliding windows, using 10,000 permutations in order to determine p - values. This analysis is a regression approach to test haplotype-trait association. The test for association then uses an F test for a specialized additive model. The strongest associated sliding window was then analyzed for haplotype specific effects. This included the calculation of Odds Ratios (OR) for each haplotype, as well as determination of PTB and term haplotype frequencies. The highest frequency haplotype was used as the baseline haplotype frequency. Only haplotypes with a frequency of 5% or more were considered for haplotype analyses and only significant haplotypes are reported.

Genes were grouped into KEGG biological pathways and Z tests were then used to determine if the total number of significant results within a pathway deviated from the expected number of significant results given the number of tests and the sample size of the dataset. These analyses used only tag SNPs ($r^2 \geq 0.60$) in order to correct for lack of independence between markers within a gene due to LD. Our analyses included all pathways with at least 5 genes and allowed for genes to be included in more than one pathway. These tests were performed both for the tests of allele associations and genotype associations separately.

Results are presented for the following analyses: 1) single locus tests of association with an arbitrary p value cutoff of $p \leq 1.00\times10^{-3}$ (this p value cutoff was used for simplicity of presentation) or either allele or genotype tests of association; 2) haplotype tests for genes with at least 1 marker with an arbitrary p value cutoff of $p \leq 1.00\times10^{-3}$ in order to stay consistent with the single locus tests of association, 3 or more markers within the genes and with at least 1/3 of the markers statistically significant at the p = 0.05 level; 3) Z-tests within

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KEGG pathways testing for deviations from expected number of significant tests (either allele or genotype) given the number of tests performed.

Results

Baseline Characteristics

As expected, significant differences between cases and controls were observed for gestational age (days) ($p < 1.00\times10^{-3}$), birth weight (g) ($p < 1.00\times10^{-3}$), APGAR 1($p < 1.00\times10^{-3}$) and APGAR 5 ($p < 1.00\times10^{-3}$) (Table 1). Other demographic measures, including measures of socioeconomic status, did not differ between cases and controls (Table 1).

Single locus

Table 2 presents the results for the single locus allele and genotype associations and ORs for the best model at each marker. Markers with the strongest associations ($p \leq 1.00\times10^{-3}$) for either maternal or fetal samples are presented. Among all of the SNPs analyzed 96 were statistically significant at the 0.05 level for either allelic or genotypic tests of association in maternal samples and 126 were statistically significant in fetal samples (Supplementary Table 2a and b).

In maternal data the most significant results ($p < 1.00\times10^{-3}$) were observed in markers from IL-15 (IL-15, OMIM #600554) and Heat Shock 70kDa Protein 1-like (HSPA1L, OMIM #140559). In IL15 marker rs10833 had significant allelic and genotypic associations (allele $p = 2.91\times10^{-4}$; genotype $p = 2.00\times10^{-3}$) with a case minor allele frequency (MAF) = 0.06 and a controls MAF = 0.18. HSPA1L marker rs2075800 had a significant genotypic association ($p = 5.93\times10^{-4}$). The most significant maternal genotypic ORs were seen for IL-15 rs10833 (OR = 0.30, 95% CI = 0.14–0.62, $p = 1.00\times10^{-3}$) and IL-1RAP rs9290936 (OR = 1.97, 95% CI = 1.29–3.00, $p = 1.00\times10^{-3}$), both are for additive models.

In fetal samples (Table 2) the most significant associations were in markers from Cytotoxic T-lymphocyte Associated Protein 4 (CTLA4, OMIM #123890), Interleukin 2 receptor beta (IL-2RB, OMIM #146710), matrix metalloproteinase 2 (MMP2, OMIM # 120360), and Tumor Necrosis Factor Alpha (TNF-α, OMIM #191160). In IL-2RB marker rs84460 and CTLA4 both had significant allelic and genotypic associations (IL-2RB allele $p = 1.37\times10^{-4}$, genotype $p = 6.29\times10^{-4}$; CTLA4 allele $p = 2.06\times10^{-4}$, genotype $p = 4.00\times10^{-3}$). The most significant genotype model for IL-2RB was an additive model for rs84460 with an OR = 2.32, (95% CI = 1.47–3.67, $p < 1.00\times10^{-3}$) and for CTLA4 the most significant model was a recessive model for rs16840252 with CC vs. (CT&TT) (OR = 3.21, 95% CI 1.61–6.40, $p = 1.00\times10^{-3}$)). For fetal genotypic analyses the largest ORs with the most significant p values were for IL-2RB rs228947 CC vs. CT,TT (OR = 2.39, 95% CI= 1.32–4.34, $p = 3.0\times10^{-3}$), although the effect size was very similar for rs84460 (OR=2.32, 95% CI=1.47–3.67, $p < 1.00\times10^{-3}$), for MMP2 rs243832 model GG vs. CG,CC (OR = 2.93, 95% CI = 1.56–5.49, $p = 1.00\times10^{-3}$), and TNF-α rs1800683 additive (OR = 0.44 (95% CI = 0.28–0.69, $p = 1.00\times10^{-3}$).

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Four of twenty-one significant markers deviated from HWE (Table 2). In maternal data deviations from HWE were seen in HSPA1L and IL-2RA. In HSPA1L marker rs2075800 deviated in cases ($p = 3.60\times10^{-5}$) and IL-2RA marker rs6602392 deviated in controls ($p = 0.04$). Two deviations were observed in fetal cases at CD14 marker rs4914 ($p = 0.02$) and PON2 rs2237585 ($p = 0.01$). The deviations from HWE in cases in HSPA1L may provide further support of an association at that marker given that deviations from HWE in cases commonly indicate stronger evidence of association. The inbreeding coefficients for cases and controls for the maternal IL2RA marker rs6602392 had opposite signs, demonstrating that the deviation from HWE was in opposite direction and not likely due to genotyping error.

A description of the markers and their proposed functions are listed in Table 3a for maternal and Table 3b for fetal data. In maternal data HSPA1L marker rs2075800 results in an amino acid change that is nonsynonymous (602 E/K). The remaining markers were promoter, intronic, exonic, and exon/intron boundary regions.

Haplotype Associations

One gene for the maternal data met the criterion for haplotype analysis, Interleukin 6 Receptor (IL6R, OMIM #147880), and eight for fetal data (Monocyte Differentiation Antigen CD14 (CD14, OMIM #158120), Cytotoxic T-lymphocyte Associated Protein 4 (CTLA4, OMIM #123890), IL-2 (OMIM #147680), IL-2RB, MMP9 (OMIM #120361), Paraxonase 2 (PON2, OMIM #602447), Prostaglandin E Receptor 3 (PTGER3, OMIM #176806), and TNF-α) met the criteria. Detailed LD structure for maternal and fetal genes for cases and controls are available in supplemental material (Supplemental Figure 1a–r). Table 4 has the results of the haplotype association analyses with only the significant haplotypes and their ORs reported for all significant ($p \leq 0.05$) haplotypes. Sliding window haplotype analyses were performed and the haplotype with the most significant global p value for a sliding window is presented.

Maternal IL-6R haplotype sliding window analyses (Table 4; Supplemental Figure 1a and b) associated intronic markers rs4075015-rs4601580-rs4845618 ($p = 0.02$) with cases. Haplotypes A-T-G (rs4075015-rs4601580-rs4845618; $p < 1.00\times10^{-3}$) and A-T-T (rs4075015-rs4601580-rs4845618; OR = 0.17 [95% CI 0.02–0.71], $p = 0.01$) were the only ones that showed association.

Examining the eight genes in fetal data (Table 4; Supplemental Figure 1c–r), the most significant haplotype ORs were seen in: CD14 (rs4914-rs2569190), CTLA4 (rs16840252-rs11571317-rs5742909), IL2 (rs2069771-rs2069776-rs2069778), IL-2RB (rs84460-rs228945-rs228947), MMP9 (rs6104420-rs3918260), and TNF-α (rs1800683-rs2229094). Among these associated haplotypes it is unclear whether MMP9 (rs6104420-3918260) and TNF-α (rs18006830-rs2229094) are driven by the haplotype effects or single markers. In MMP9 (rs6104420-3918260) G-C (OR = 2.27, 95% CI 1.31–3.91, $p = 2.00\times10^{-3}$) had the strongest haplotype association and in TNF-α A-C (rs1800683-rs2229094; OR = 0.27, 95% CI 0.20–0.68, $p = 1.00\times10^{-3}$) had the strongest association. The haplotype associated in MMP9 include promoter and intron SNPs and within TNF-α a promoter (rs1800683) and coding marker (rs2229094; nonsynonymous amino acid change 13 R/C) are included.

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Haplotypes in PON2, PTGER3 and TNF-α haplotype 2 all had significance levels no smaller than p = 0.02 for specific haplotypes.

Evidence of pathway involvement

All 130 genes were grouped into KEGG biological pathways (Table 5) to assess whether specific pathways have an excess of significant findings. In maternal data, only the cytokine pathway had more significant genotype and allele associations than expected (Table 5).

Globally, across all single locus allelic analyses, fetal samples had more significant allelic associations than would be expected given the number of tests performed. The cytokine pathway was significant for both allele and genotype associations for fetal data. However, for fetal samples the hematopoietic cell lineage, the Jak-STAT signaling, and the Toll-like receptor pathways were also significant.

Comment

The present study examined candidate genes from multiple PTB pathways in an effort to elucidate the genetic predisposition in African American PTB. Multiple interesting single locus and haplotype associations in both maternal and fetal data were observed and confirmed previously established candidates. In maternal samples the strongest associations for both single locus allele, genotype, and haplotype tests were in genes from infection and inflammatory response preterm birth pathways that include markers from HSPA1L, IL-15, IL-1RAP, IL-2RA, IL-6R, and TNFRSF1B (TNFR2). Similarly, in fetal samples, the most significant associations were observed in infection and inflammatory response pathways, including, among others, significant associations in IL-2, IL-2RB, MMP2, MMP9, and TNF-α. Although none of these associations were significant after corrections for multiple testing, the multiple significant associations found among genes from the infection and inflammatory pathways strongly suggest the importance of this pathway in determining African American preterm births. Additionally and as expected, these data indicate that no single gene explains risk in African Americans, but rather locus heterogeneity appears to exist for multiple genes from this pathway as expected in a complex phenotype such as PTB. These findings are consistent with the documented elevated rates of infection seen in African American PTB [29;30].

Single locus and haplotype associations revealed several findings that may indicate functional relevance of the genes. One haplotype was associated in maternal data (IL-6R) and nine were associated in fetal data (one each in CD14, CTLA4, IL-2, IL-2RB, MMP9, PON2, PTGER3, and two in TNF-α). One of the associated haplotypes in TNF-α (rs1800683-rs2229094-rs1799964-rs1800629) included a well established PTB candidate -308 (rs1800629) [31–33]. TNF-α -308 also had a significant main effect (allele p = 0.01; genotype p = 0.02) in fetal data, but it did not associate in maternal DNA even at a marginal level.

Replication of TNF-α -308 associations in maternal data has been inconsistent across studies [34]. Despite this, elevated concentrations of TNF-α have been observed in PTB [35;36] supporting a role for this gene in African Americans. It may be that fetal rather than

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maternal tissues contribute to the elevated TNF-α concentration. A large cohort study by Aidoo *et al.* 2001 associated TNF-α -308 with fetal genotype in PTB, malaria, and with increased fetal mortality [37]. Their results suggest that -308 may have a genetic effect for fetal genotype rather than maternal. Our results are consistent with a fetal -308 single marker genotype effect as well as a haplotype effect.

Although we did not directly perform any tests of maternal and/or fetal genetic interactions, we did observe several indirect associations in genes and their receptors across maternal and fetal data that may indicate interactions may be occurring between maternal and fetal genotypes. Specifically, in maternal samples IL-2RA and TNFR2 associated with PTB and in fetal samples IL-2, IL-2RB, and TNF-α associated with PTB.

Several associations were observed in fetal data. The majority of the genes significant in fetal data are expressed in fetal tissue and are related to infection and/or inflammation during pregnancy. Studies examining CD14[38–40], CTLA4[41], IL-2[42], MMP2[43;44], MMP9[45;46], PON2[47], PTGER3[48], and TNF-α [42] have shown the expression of these genes in fetal tissues (amniochorion). These data and our associations together support a genetic basis for the observed altered expression of these genes in fetal tissues in PTB.

Our pathway analyses were performed to assess biological and not hypothesized PTB pathways, doing so identified the cytokine/infection/inflammation pathway as the most associated pathway in African Americans. This is a very promising finding given that it is consistent with a higher rate of infection in African Americans during pregnancy. Further studies of these findings will, however, be necessary since the biological interpretation of these results is unclear as to whether the type of infection is critical.

In addition we observed an unexpectedly large number of associations with fetal genotypes but not with maternal genotypes, suggesting that the fetal genetic contribution to PTB exceeds the maternal contribution in African Americans. This finding is in contrast to that of Wilcox et al who suggest that fetal contribution to preterm is not strong because of limited paternal contribution in their analyses[49]. However, this study was performed in a very different population than ours (Norwegians vs. African Americans) and may be related to population specific risks. Our findings are supported by other studies that show paternal race is associated with PTB, and specifically, that increased risk is associated with the father being African American, implicating a fetal contribution in this population[50;51].

In conclusion, we conducted a gene-centric association study on PTB and found several interesting associations with PTB in African Americans. We also identified the cytokine infection and inflammatory response pathway as having the largest proportion of statistically significant associations in both maternal and fetal data. Although sample size is a limitation to our study design, given the patterns of associations observed within genes and by pathway, it is clear that several of the findings are consistent with established literature. We also note that although we increased our sensitivity by using a non-continuous outcome and removing borderline gestational ages, this may increase our chances of a type I error. These studies, although not conclusive did identify several interesting candidates for follow-up in replicate studies.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical and demographic information

Variable	Case (N=82) Mean (SD), Median[IQR], Count	Control (N=197) Mean (SD), Median[IQR], Count	P-Value[1]
Gravidity (number of previous pregnancies)	2[1–6]	2 [1–13]	0.96
Gestational Age (days)	242 [154–255]	273 [257–290]	<0.001
Gestational Weight (g)	2240 [462–3782]	3190 [1952–4517]	<0.001
APGAR 1	8 [1–9]	8 [3–9]	<0.001
APGAR 5	9 [6–10]	9 [7–10]	<0.001
Maternal Age (yrs)	25.32 (5.53)	25.22 (5.29)	0.88
School/Education(yrs)	12[6–12]	12[6–12]	0.22
Smoking (%)	36.67%	21.25%	0.08
Infection (% Cases)	10.84%		
Income[2]			0.90
<$15000	43	92	
$15000–30000	15	44	
$30000–50000	16	35	
>$50000	8	21	
Insurance[2]			0.65
TN Care	58	122	
Commercial	8	21	
Managed Care	0	1	
Through Employer	16	49	
Marital Status[2]			0.67
Divorced	3	4	
Married	24	53	
Single	49	114	

Means are reported with standard deviations reported in parentheses and medians are reported with interquartile ranges in brackets

[1]P-values compare cases and controls using a Mann Whitney rank sum test.

[2]P-A α^2 or Fisher's Exact test was used to compare cases to controls

Means are reported with standard deviations reported in parentheses and medians are reported with interquartile ranges in brackets

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Table 2

Single locus association results and genotypic ORs

Population	*Gene(s)*	SNP rs#	Allele	Case Freq.	Control Freq.	Case v Control P		Model	OR	95% CI	Model P
						Allele	Genotype				
Maternal	*HSPA1L*	rs2075800[1]	A	0.07	0.09	0.61	5.93×10^{-4}	AAvAG&GG	10.70	1.18–97.39	0.04
	IL15	rs10833	A	0.06	0.18	2.91×10^{-4}	2.00×10^{-3}	additive	0.30	0.14–0.62	1.00×10^{-3}
	IL1RAP	rs9290936	T	0.37	0.23	1.53×10^{-3}	0.01	additive	1.97	1.29–3.00	1.00×10^{-3}
	IL2RA	rs6602392[2]	A	0.13	0.23	4.46×10^{-3}	0.03	additive	0.52	0.31–0.86	0.01
	IL6R	rs4553185	C	0.48	0.61	4.49×10^{-3}	0.01	additive	1.69	1.15–2.50	0.01
	TNFRSF1B	rs5746053	A	0.20	0.11	0.01	3.00×10^{-3}	additive	2.04	1.20–3.47	9.00×10^{-3}
Fetal	*CD14*	rs4914[1]	G	0.10	0.03	2.51×10^{-3}	0.02	additive	2.77	1.32–5.82	0.01
	CTLA4	rs16840252	T	0.10	0.25	2.06×10^{-4}	4.00×10^{-3}	CCvCT&TT	3.21	1.61–6.40	1.00×10^{-3}
	CTLA4	rs231777	T	0.16	0.29	2.52×10^{-3}	0.01	additive	0.48	0.28–0.80	0.01
	IL2	rs2069771	T	0.16	0.30	1.75×10^{-3}	0.01	additive	0.45	0.27–0.76	0.01
	IL2RB	rs84460	C	0.25	0.44	1.37×10^{-4}	6.29×10^{-4}	additive	2.32	1.47–3.67	$<1.00\times10^{-3}$
	IL2RB	rs228947	T	0.17	0.31	8.80×10^{-4}	0.01	CCvCT&TT	2.39	1.32–4.34	3.00×10^{-3}
	IL2RB	rs3218315	T	0.18	0.30	3.81×10^{-3}	0.02	TTvCT&CC	2.37	1.30–4.34	0.01
	IL2RB	rs228954	G	0.42	0.57	2.47×10^{-4}	0.01	additive	1.80	1.20–2.71	4.70×10^{-3}
	IL2RB	rs2281094	T	0.14	0.26	3.46×10^{-3}	0.02	CCvCT&TT	2.19	1.18–4.06	0.01
	MMP2	rs243832	G	0.40	0.55	3.54×10^{-3}	2.71×10^{-3}	CCvCG&GG	2.93	1.56–5.49	1.00×10^{-3}
	MMP9	rs3918260	C	0.27	0.14	1.37×10^{-3}	3.30×10^{-3}	additive	2.14	1.31–3.50	2.50×10^{-3}
	PON2	rs2237585[1]	T	0.45	0.58	0.01	2.70×10^{-3}	TTvCT&CC	0.29	0.13–0.64	2.00×10^{-3}
	PON2	rs2299267	G	0.22	0.12	3.92×10^{-3}	0.01	additive	2.04	1.20–3.47	4.0×10^{-3}
	PTGER3	rs4147115	A	0.39	0.53	4.75×10^{-3}	0.01	additive	0.55	0.36–0.85	6.30×10^{-3}
	TNF-a	rs1800683	A	0.35	0.51	6.33×10^{-4}	4.06×10^{-3}	additive	0.44	0.28–0.69	3.00×10^{-4}

[1] maternal cases deviated from HWE at rs2075800 ($p = 3.60\times10^{-5}$) and fetal cases deviated at rs4914 (p = 0.02) and rs2237585 (p = 0.01)

[2] maternal controls deviated from HWE at rs6602392 (p = 0.04)

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Table 3

Markers with strongest associations

a. Maternal

Gene Name	*Gene Code*	Band[1]	dbSNP rs#	Role	Amino Acid Change	KEGG Pathway
Heat Shock 70kDa Protein 1-like	*HSPA1L*	6p21.33	rs2075800	Coding exon	602 E/K	MAPK signaling pathway, antigen processing and presentation
Interleukin 15	*IL15*	4q31.21	rs10833	Exon	-	Cytokine-cytokine receptor interaction, Jak-STAT signaling
Interleukin 1 Receptor Accessory Protein	*IL1RAP*	3q28	rs9290936	Intron	-	Cytokine-cytokine receptor interaction, apoptosis
Interleukin 2 Receptor, Alpha	*IL2RA*	10p15.1	rs6602392	Intron	-	Cytokine-cytokine receptor interaction, Jak-STAT signaling, hematopoietic cell lineage
Interleukin 6 Receptor	*IL6R*	1q21.3	rs4553185	Intron	-	Cytokine-cytokine receptor interaction, hematopoietic cell lineage, Jak-STAT signaling
Tumor Necrosis Factor Receptor Superfamily, Member 1B	*TNFRSF1B*	1p36.22	rs5746053	Intron, Exon/ intron boundary	-	Apoptosis, cytokine-cytokine receptor interaction, MAPK signaling pathway, adipocytokine signaling pathway

b. Fetal

Gene Name	*Gene Code*	Band[1]	dbSNP rs#	Role	Amino Acid Change	KEGG Pathway
Monocyte Differentiation antigen CD14	*CD14*	5q31.3	rs4914	Coding exon	367 L/L	Regulation of actin cytoskeleton, pathogenic Escherichia coli infection - EPEC, Hematopoietic cell lineage, pathogenic Escherichia coli infection - EHEC, MAPK signaling pathway, toll-like receptor signaling pathway
Cytotoxic T-lymphocyte Associated Protein 4	*CTLA4*	2q33.2	rs16840252	Promoter	-	T cell receptor signaling pathway, cell adhesion molecules (CAMs)
			rs231777	Intron	-	
Interleukin 2	*IL2*	4q27	rs2069771	Intron	-	Jak-STAT signaling pathway, type I diabetes mellitus, T cell receptor signaling pathway, cytokine-cytokine receptor interaction
Interleukin 2 Receptor, beta	*IL2RB*	22q13.1	rs84460	Intron	-	Cytokine-cytokine receptor interaction, Jak-STAT signaling pathway
			rs228947	Intron	-	
			rs3218315	Intron	-	
			rs228954	Intron	-	
			rs2281094	Intron	-	

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b. Fetal

Gene Name	*Gene Code*	Band[1]	dbSNP rs#	Role	Amino Acid Change	KEGG Pathway
Matrix Metalloproteinase 2	*MMP2*	16q12.2	rs243832	Intron (boundary)	-	GnRH signaling pathway, leukocyte transendothelial migration
Matrix Metalloproteinase 9	*MMP9*	20q13.12	rs3918260	Intron (boundary)	-	
Paraoxonase 2	*PON2*	7q21.3	rs2237585	Intron	-	Gamma-Hexachlorocyclohexane degradation, bisphenol A degradation
			rs2299267	Intron	-	
Prostaglandin E Receptor 3	*PTGER3*	1p31.1	rs4147115	Intron	-	Calcium signaling pathway, euroactive ligand-receptor interaction
Tumor Necrosis Factor - Alpha	*TNF-α*	6p21.33	rs1800683	Promoter	-	Hematopoietic cell lineage, Fc epsilon RI signaling pathway, type II diabetes mellitus, cytokine-cytokine receptor interaction, natural killer cell mediated cytotoxicity, toll-like receptor signaling pathway, T cell receptor signaling pathway, MAPK signaling pathway, Apoptosis, adipocytokine signaling pathway, TGF-beta signaling pathway, type I diabetes mellitus

[1] NCBI build 35.1

[2] Gene Ontology and KEGG pathway information obtained from SNPper (http://snpper.chip.org) and KEGG gene ontology browser

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Table 4

Haplotype frequencies and OR for strongest sliding window

Population	*Gene(s)*	Markers	Global P[1]	Haplotype	Frequency		OR	95% CI	P
					Case	Control			
Maternal	*IL6R*	rs4075015-rs4601580-rs4845618	0.02	T-T-T	0.30	0.31	1.00	-	-
				A-T-G	0.07	$<10^{-3}$	-	-	$<10^{-3}$
				A-T-T	0.02	0.08	0.17	0.02–0.71	0.01
Fetal	*CD14*	rs4914-rs2569190	0.01	C-G	0.49	0.60	1.00	-	-
				G-G	0.11	0.03	3.91	1.58–9.71	1.00×10^{-3}
	CTLA4	rs16840252-rs11571317-rs5742909	3.00×10^{-3}	C-T-C	0.88	0.73	1.00	-	-
				T-T-C	0.10	0.22	0.37	0.18–0.71	1.00×10^{-3}
	IL2	rs2069771-rs2069779-rs2069778	0.01	C-C-C	0.82	0.66	1.00	-	-
				T-C-C	0.15	0.30	0.39	0.22–0.68	$<10^{-3}$
	IL2RB	rs84460-rs228945-rs228947	4.00×10^{-4}	T-A-C	0.56	0.42	1.00	-	-
				C-G-T	0.16	0.26	0.45	0.25–0.80	4.00×10^{-3}
				C-G-C	0.09	0.17	0.40	0.18–0.80	0.01
	MMP9	rs6104420-rs3918260	0.02	A-T	0.67	0.76	1.00	-	-
				G-C	0.24	0.12	2.27	1.31–3.91	2.00×10^{-3}
	PON2	rs2237585-rs11976060	2.00×10^{-3}	T-C	0.55	0.42	1.00	-	-
				C-C	0.45	0.58	0.61	0.40–0.93	0.02
	PTGER3	rs2050066-rs6424414-rs2300167-rs6678886	2.00×10^{-3}	T-C-T-A	0.40	0.39	1.00	-	-
				T-T-T-A	0.17	0.29	0.59	0.33–1.06	0.06
				T-T-T-G	0.11	0.05	2.12	0.92–4.75	0.05
	TNF-α	rs1800683-rs2229094	2.00×10^{-3}	G-T	0.59	0.45	1.00	-	-
				A-T	0.21	0.25	0.66	0.38–1.11	0.10
				A-C	0.13	0.27	0.37	0.20–0.68	1.00×10^{-3}
		rs1800683-rs2229094-rs1799964-rs1800629	2.00×10^{-3}	G-T-T-G	0.39	0.33	1.00	-	-

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Population	*Gene(s)*	Markers	Global P[1]	Haplotype	Frequency		OR	95% CI	P
					Case	Control			
				A-C-C-G	0.11	0.19	0.51	0.25–1.00	0.04
				A-C-T-G	0.02	0.07	0.27	0.05–0.93	0.03

[1] Global P is the p-value for the haplotype sliding window

[*] Only haplotypes with frequencies of 5% in at least one status group and with a significant OR are presented.

[*] ORs are calculated comparing each haplotype to the highest frequency haplotype.

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Table 5

Significant results by KEGG pathway using only tags

KEGG Pathway (tags with $r^2 \geq 0.60$)	# Genes	#SNPs in pathway[1]	Population	
			Maternal	Fetal
Apoptosis	12	139		
Arachidonic acid metabolism	5	72		
Compliment and coagulation cascade	6	47		
Cytokine-cytokine receptor interactions	31	357	AG (17/18)	AG (36/26)
Focal adhesion	6	97		
Hematopoietic cell lineage	12	144		A (14/NA)
Jak-STAT signaling pathway	14	172		AG (25/17)
MAPK signaling pathway	18	136		
Neuroactive ligand-receptor interaction	12	160		
T cell receptor signaling pathway1	12	75		AG (12/11)
Toll-like receptor signaling	14	65		
Type I diabetes mellitus	7	48		
Other (Genes not in other pathways)	55	457		

[1] Genes can be involved in more than one pathway and as a result SNPs can overlap with other pathways. (A/G)indicates the total number of significant associations observed in a pathway for allelic (A) or genotypic (G) tests of association. If one of the two sets of comparisons not significant NA is used for the non-significant one.

G - indicates a statistically significant genotypic association

A- indicates marginally significant pathway for allelic association